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Study of spectral response of plant pigments for remote sensing applications

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Abstract : A common aspect of different applications of remote sensing to plant sciences is to study the visible and infrared radiation reflected from vegetation canopies. The reflectance is governed by chlorophyll and other plant pigments and also by the presence of water, other tissues and the ground itself. Therefore the standardization of spectral response of the pigments is very important. The present work characterizes the visible spectral response of chlorophyll and carotenoids at different growth stages of the leaves and justifies its capability to monitor maturity stages of vegetation. Only the visible region of spectral band is chosen because that is mainly concerned with the property of the vegetation itself. Instead of the amplitude of the peak reflectance band, as considered conventionally, the wavelength corresponding to the peak reflectance is taken into account. To indicate crop maturity stages like "greenness" and "yellowness", fuzzy operations over reflectance data are suggested so as to simulate human reasoning.

Keywords : Remote sensing, fuzzy logic.

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1. Introduction

Remote sensing in plant sciences involves the study of visible and infrared radiation reflected from vegetation canopies. The presence of chlorophyll and other pigments significantly influences the reflectance. Therefore the standardization of plant pigment response to different wavelengths of radiation is of much importance. Conventionally reflectance is measured for red and infrared bands and vegetation vigor is numerically defined in terms of amplitude of these values, *e.g.*, normalized difference vegetation index (NDVI). Recently a trend towards hyperspectral remote sensing is being observed [1-3] that encourages fine resolution in wavelength and standardization of spectral response. Keeping in mind the above, the present work investigates whether only the visible spectral response of plant pigments can be used as a biological system to monitor crop maturity stages. It characterizes the visible spectral response of chlorophyll and carotenoids extracted at different maturity stages of the leaves. The visible region is preferred because infrared reflectance may be contaminated by ground reflectance but visible reflectance is the property of the vegetation itself. In addition to the spectral range of study, the present work differs from conventional method in two other points. Instead of peak reflectance amplitude, the wavelength range corresponding to normalized peak reflectance is made into use. Also, fuzzy logic is used to simulate human reasoning in defining crop maturity stages.

2. Experimental

The apparatus for spectroscopic study was set up by assembling discrete instruments in the laboratory. It consisted of collimated white light dispersed by prism so that on rotating the prism a narrow light beam of continuously variable wavelength was obtained. The wavelength value could be read from a graduated scale of 1 nm resolution. The sample was placed at 45° angle in front of the beam and the reflected light was sensed by photomultiplier tube calibrated in terms of current. Samples were prepared in the following way.

Pigments were extracted from common gourd leaves in both tender-matured (green) and senescent (yellow) stages. Standard chemical dissolving was done with a mixture of petroleum ether, methanol and benzene in

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(45 : 15 : 5) ratio. The following three types of samples were prepared from the solution of leaf extract.

(i) Chlorophyll-carotenoid mixture was deposited on glass plate by dip and dry method.

(ii) Chlorophyll and xanthophyll were separated on thin layer chromatographic (TLC) paper by housing the liquid on the paper within saturated vapour of petroleum ether-acetone (19 : 1) mixture for 24 hours.

(iii) Pieces of fresh leaf were also used as samples by tightly sandwiching between two thin glass cover slips.

The purpose was not to obtain the absolute value of reflectance in any case. The relative change in reflected light intensity with wavelength was to be studied. So no reference object was needed. The reflectance values in terms of current were normalized with respect to the peak value and plotted against wavelength as shown below.

3. Results and analyses

The normalized reflectance *vs* wavelength curves for tender-matured and senescent fresh leaves are shown in Figure 1. The wavelengths corresponding to peak reflectance, as shaded in the figure are summarized in Table 1. Conventionally the reflectance for the whole visible range is scanned in only two or three bands of wavelength without fine resolution between green and yellow. Changes in reflectance magnitude are compared between visible and infrared range for tender and matured plants. However, a living leaf contains mesophyll tissue that have significant influence on infrared scattering [1]. But as obvious from the figure, it does not hamper the reflectance in visible range. The green and yellow reflectances are separated by at least 10 nm that can be distinguished by an optical sensor.

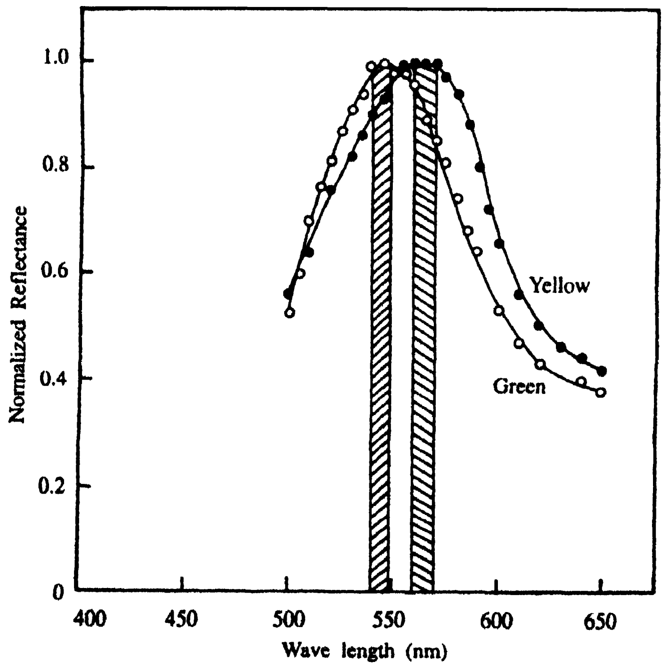

Figure 1. Reflectance curves for fresh leaves, tender-matured (green) and senescent (yellow).

Figure 2 consists of similar curves for pigment extracts deposited on glass plate. Here the effect of mesophyll tissue is eliminated. The expected situation is majority of chlorophyll in tender-matured stage and dominance of decomposed chlorophyll and xanthophyll at senescent stage. Each has characteristic peak reflectance for distinct ranges of wavelength. Other standard results are also incorporated for comparison.

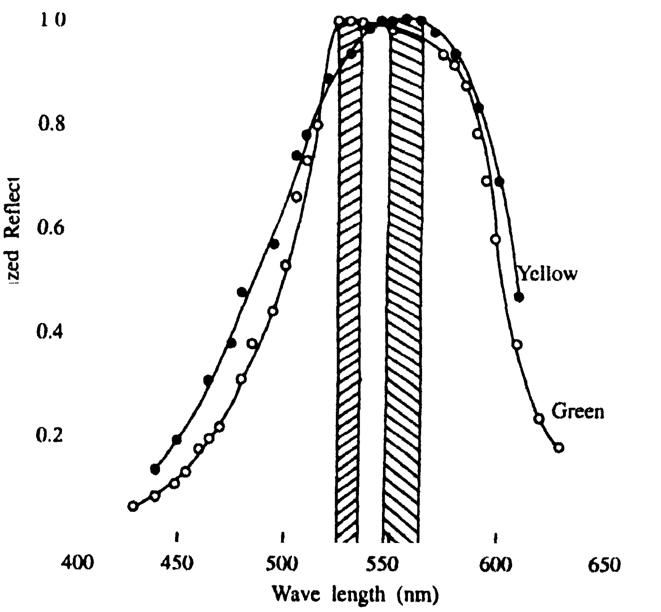

Figure 2. Reflectance curves for chlorophyll-carotenoid mixture as extracted from tender-matured (green) and senescent (yellow) leaves.

Figure 3 exhibits the spectral response of chlorophyll (green) and xanthophyll (yellow) separated on TLC paper. Also the reflectance was measured after open-air degradation of the TLC deposition for three months as shown in Figure 4. It is seen that even after such an exaggerated degradation, the peak reflectance exhibits partial separation for the above two pigments. Thus, it may be concluded that the distinction in wavelength of peak visible reflectance from plant pigments can be used as a sensitive tool for estimation of vegetation vigor.

Any parameter like soil water content, nitrogen deficiency, plant health *etc.* can be revealed through changes in chlorophyll reflectance. The present work, however studies only the effect of natural senescence that is associated with destruction of chlorophyll and consequent prominence of xanthophyll. Instead of reflectance magnitude ratios at different wavelength bands, the wavelength corresponding to peak reflectance can be used as a vegetation index. It makes the process more distinct and suitable for hyperspectral remote sensing.

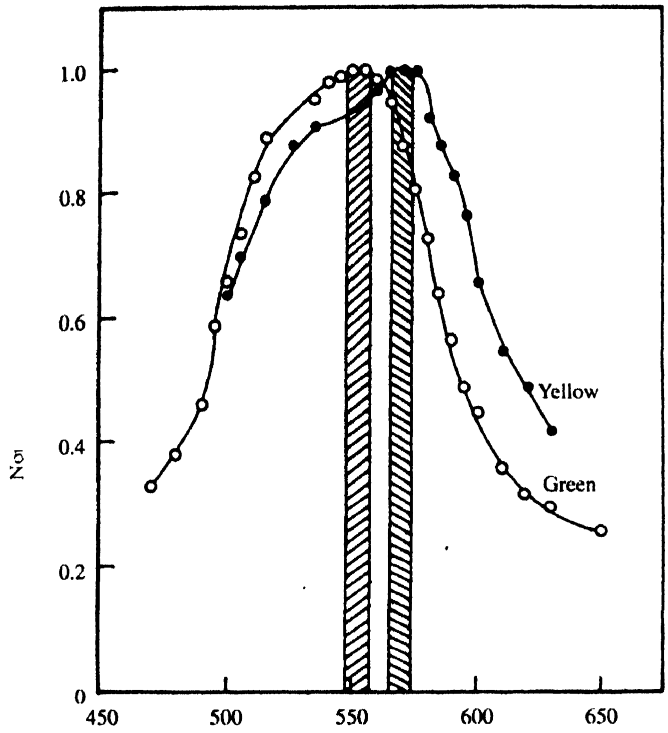

Figure 3. Reflectance curves for fresh TLC separations.

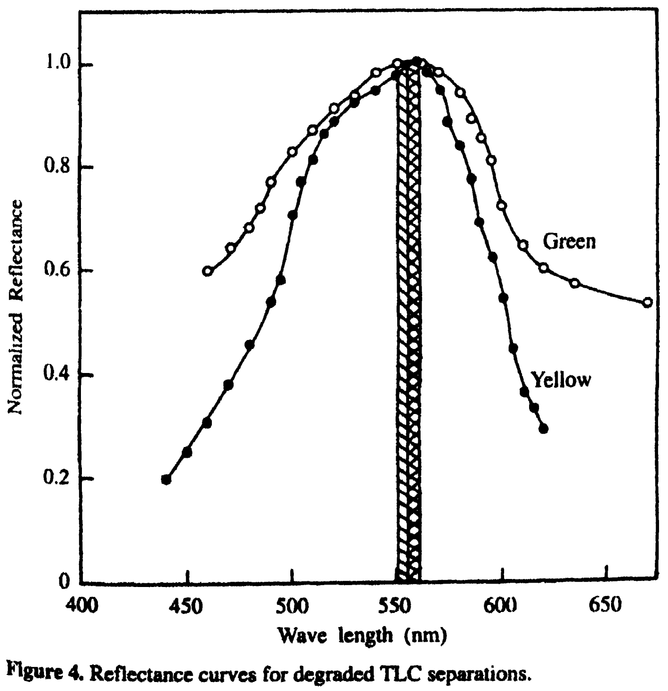

Figure 4. Reflectance curves for degraded TLC separations.

Table 1. Wave length(nm) range corresponding to peak reflectance/ minimum absorbance for different samples.

Present work	Peak green	Peak yellow
(i) Fresh leaf	540–550	560–570
(ii) Leaf extract	525–535	550–565
(iii) TLC (fresh)	545–555	565–575
(iv) TLC degraded)	550–560	555–560
Ref [4]		
(i) Leaf extract	Absorption min. Green 520–550	Absorption min. Yellow 560–575
(ii) Chlorophyll a and b	Around 500	—

Reflectance *vs* wavelength data like the above, obtained in real field should be properly analyzed for having idea on 'greenness' or 'yellowness' of the vegetation. Comparison of the reflectance peak positions may be reliable for an ideal band spectrum. But the remotely sensed data represent natural phenomena that rarely have crisp boundaries. A pixel of the digital image corresponds to a part of land area that need not produce reflectance maxima/minima within sharp specified limits. Therefore fuzzy methodology is becoming popular in such applications to account for the degree of variation. The basic idea is to classify each pixel into multiple categories based on estimated membership in each class. Two different examples are cited where fuzzy models have been applied to analyze soil configuration [5] and urban area classification [6]. The present work is a straightforward laboratory model. However, a realistic data set of similar type can be analyzed with fuzzy logic as explained below.

The human eye can easily compare two colours and distinguish between green and yellow of both the fresh leaves and of TLC separations. But in terms of reflectance, continuous spectra are obtained with separate peaks and finite overlapping. The peak positions are also not fixed as noted from Table 1. In such a case the decision on 'greenness' or 'yellowness', that is to be made electronically or computationally becomes convenient with fuzzy OR($\vee$) and fuzzy AND($\wedge$) operations. For each wavelength, the corresponding normalized reflectance is considered to be the membership value (μ) varying between 0 and 1. Now if a certain green reflectance variation (G) is compared with another green reflectance variation (G_1) obtained later, then it is obvious that

$$G \vee G_1 = \max\,[\mu(G),\ \mu(G_1)]$$

values steadily fall with increase in wavelength. Such a continuous fall indicates "greenness". But if the green is compared with an yellow reflectance variation (Y), then

$$G \vee Y = \max\ [\mu(G),\ \mu(Y)]$$

exhibits a decrease, then increases again. The presence of such an intermediate fall indicates 'yellowness'. The above arguments are exemplified in Table 2 taking a few data from different sets in the vicinity of the peaks.

Table 2. Examples of fuzzy operations with the present data.

Operations	Wavelength (nm) and corresponding normalized reflectance values					
	540	545	550	555	560	565
$G \vee G_1$	1.0	1.0	1.0	1.0	0.99	0.95
$G \vee Y$	1.0	1.0	0.98	0.99	1.0	1.0
$G \vee G_1$	0.98	0.99	0.98	0.98	0.96	0.89
$G \vee Y$	0.90	0.93	0.98	0.98	0.96	0.89

A third possibility may occur if, in any case the peak reflectance of green and that of yellow almost overlap. Then fuzzy OR falls steadily leading to false conclusion of greenness. So fuzzy AND is to be used as a second test. In this case, fuzzy AND undergoes a peak value which is not possible between two green or two yellow variations. This is also indicated in Table 2 with a suitable set of data.

4. Summary

Visible spectral response of plant pigments has been justified as a biological system to monitor crop maturity stages. The vegetation vigor is defined not in terms of reflectance amplitude, but in terms of wavelength corresponding to peak reflectance. The process suggested is to record the wavelength *vs* normalized reflectance data for the vegetation at different growth stages and to execute fuzzy OR and AND operations between two sets of data. The existence of both an intermediate rise in fuzzy AND and intermediate fall in fuzzy OR indicates yellowness, otherwise greenness may be concluded.

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